FINAL REPORT

INFLUENCE OF BROAD BAND UV-B ON PHYSIOLOGY AND BEHAVIOR OF BENEFICIAL AND HARMFUL INSECTS

Dora K. Hayes
Chemical and Biophysical Control Laboratory
Agricultural Environmental Quality Institute
Beltsville Agricultural Research Center
Beltsville, Maryland 20705

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Project Officer:

R. J. McCracken
Agricultural Research, Science and Education Administration
U.S. Department of Agriculture
Washington, D.C. 20250

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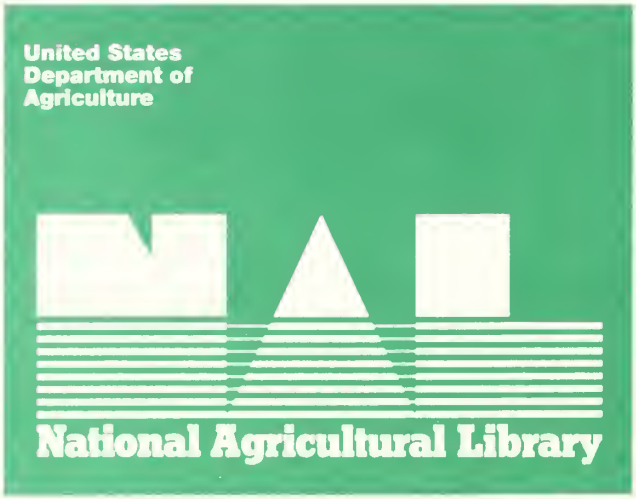
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FOREWORD

An increase in UV-B may be beneficial or harmful, depending upon the effects of such an increase on pest species of insects and their host plants. The task of the Chemical and Biophysical Control Laboratory was to investigate the influence of broad band UV-B on physiology and behavior of beneficial and harmful insects in the following ways:

(a) Insects, where technology was available for rearing, were used to determine the direct effect of UV-B on selected developmental stages and on interactions of insects with crops of economic importance.

(b) Effects of UV-B on metamorphosis, biological rhythms, and diapause were investigated and the possibility that photorepair occurred was briefly examined.

The investigators examined the direct effects of UV-B by conducting life span determinations and by measuring egg hatch, life span and fucundity in several generations of face flies and house flies. Pigment formation after UV-B exposure indicated "sunburn". Host plant insect interaction studies were conducted using soybeans and bush beans as hosts for the tobacco budworm. Cotton plants were reared to determine effects of UV on pink bollworm hatch. Effects of UV-B on life span of honey bees was measured. Other physiological and biochemical effects could have been subtle. Effects of UV-B on metamorphosis have already been mentioned. The capability for metamorphosis may have been related to pigment formation and the capacity to diapause. Photorepair *in vivo* was only touched upon; enzymatic activity and biogenic amine levels were examined; the rhythms in oxygen utilization provided a nondestructive technique for examining effects of UV-B on insects.

ABSTRACT

Our task was to study the influence of broad band UV-B on the physiology and behavior of beneficial and harmful insects. This study was approached at two levels: the first being to determine direct effects which could be observed by determining mortality, observing increased pigmentation or darkening, etc., and the second being to determine the physiological and biochemical effects which require some further manipulation of whole or macerated insects. To determine the direct effects, our laboratory has investigated the following: life span, egg hatch and adult emergence, pigment formation (by gross observation), and host-plant interaction. The physiological and biochemical effects investigated were as follows: metamorphosis, enzymatic activity, pigment formation (detected biochemically), and rhythm using oxygen utilization as an indicator.

Hatch of pink bollworm eggs, Pectinophora gossypiella (Saunders), was decreased by at least 6 hr exposure to an irradiance level of 3.61 mWm^{-2} BUV (0.37 Wm^{-2} Abs) radiant power between 280-320 nm. Life span of the pink bollworms which hatched from eggs irradiated for 1 hr with UV-B was decreased. Exposure throughout life of insects exposed during the egg stage to a regimen with no darkness prolonged life of irradiated insects over those maintained in LD 16:8 in which the photoperiodic regime was manipulated. Diapause was potentiated in the pink bollworm in one test but not in a second after irradiation of eggs for 1 hr with 3.61 mWm^{-2} BUV (0.37 Wm^{-2} Abs) at 280-320 nm.

Eclosion of face fly, Musca autumnalis De Geer, pupae which have a calcereous non-melanized puparium was decreased by irradiating with UV-B 6 hr per day for 3-4 days. Fecundity and/or hatch of eggs from those face flies which emerged was also decreased. No such effects were found on house fly pupae which are melanized. Life span in young honey-bee, Apis mellifera L., workers was not altered, nor were ultradian rhythms and level of O_2 uptake altered by exposure to UV-B.

Melanization in vivo and/or in vitro in larvae of the codling moth, Laspeyresia pomonella (L.), face fly and tobacco budworm, Heliothis virescens (F.), was increased by UV-B. No change in metabolism of 3-hydroxykynurenine was found. Oxygen uptake of last instar codling moth larvae was increased by 1 hr of UV-B irradiation, and ultradian rhythms were obliterated. In addition, in these insects, release after treatment with triglycine or tetraglycine of material absorbing at 3, 6.3, and 7.1μm was greatly enhanced by irradiation with UV-B.

CONTENTS

LIST OF ILLUSTRATIONS

Number

LIST OF ABBREVIATIONS

AEL	--Agricultural Equipment Laboratory
DOPA	--3,4-dihydroxyphenylalanine
IL	--Instrumentation Laboratory
3-OHK	--3-hydroxykynurenine
PSL	--Plant Stress Laboratory
UV-B	--Ultraviolet-B 280-320 nanometer portion of electromagnetic spectrum
hr	--hour
LD	--light: dark ratio, expressed in hours
PBW	--pink bollworm

ACKNOWLEDGMENTS

The assistance of the Plant Stress Laboratory, Plant Physiology Institute, AR, SEA, in providing UV-fixtures, supports, and plants is gratefully acknowledged. Without the assistance of the Plant Physiology Institute Chairman, studies on hatching of pink bollworm eggs could not have been done. The Agricultural Equipment Laboratory, Plant Physiology Institute, and the Instrumentation Laboratory, Market Quality Research Institute, furnished assistance in determining intensities and spectral distributions of UV light.

Insects were provided by AR, SEA, laboratories at Yakima, Washington, Brownsville, Texas, and Plant Protection Institute, BARC, Beltsville, Maryland.

The assistance of Thor Lehnert, of the Bioenvironmental Bee Laboratory, Plant Protection Institute, AR, SEA, was invaluable in determining the effects of UV-B on bees.

INTRODUCTION

The primary effort in the AR, SEA, laboratories at Beltsville was directed toward developing techniques for irradiating biological samples with UV-B , which is well characterized in terms of energy levels and spectral distribution.

Instrumentation Laboratory (IL) (Task X-XIII), Agricultural Equipment Laboratory (AEL) (Tasks XIV-XVI), and the Plant Stress Laboratory (PSL) (Tasks I=IV) have reported on equipment for determination of energy levels, spectral distributions, and standardization of conceptual ways in which exposures are expressed. The work reported here on insects suggests what may or may not happen out-of-doors if levels of UV-B radiation are increased.

In some instances, intensities are reported in "suns." These values are approximate numbers and in most cases, such intensities were provided to us early in the testing process. Later data are reported in biologically effective UV-B in mWm^{-2} BUV using the weighting function $A\Sigma 9$ described in the AEL final report. Absolute values for UV are also given in Wm^{-2}.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

a. Hatch of pink bollworm eggs was decreased by at least 3 hr exposure to UV-B. Eclosion of face fly pupae is decreased by three or four 6 hr exposures to UV-B; eclosion of house fly pupae is not affected. As in higher life forms, a pigmented exterior protects the organism from damage that is easily discerned. No effects on fertility of house flies were observed, but UV-B irradiation of pupae decreased fertility and fucundity of face fly adults.

b. Gross observations indicated increased pigment formation in larvae of the codling moth and pupae of the face fly after exposure to UV-B. *In vitro* increase in rates of melanization in homogenates of irradiated insects in the presence or absence of 3,4-dihydroxyphenylalanine might be paralleled *in vivo*.

c. UV-B irradiation of tobacco budworm larvae on soy beans and bush beans had little effect.

d. Metamorphasis from larva to pupa was delayed in the codling moth by irradiation with UV-B.

e. No effects on 3-hydroxykynurenine metabolism but some effects on 3,4-dioxyphenylalanine (DOPA) metabolism were observed after irradiation with UV-B. This kind of activity may be related both to biogenic amine metabolism and to the capacity to diapause. Amines such as dopamine may be involved in expression of intracellular effects of UV-B irradiation.

f. No effects on induction or prevention of diapause in the pink bollworm as a result of exposure of eggs to UV-B were noted.

g. Capacity for *in vivo* photorepair may exist in insects; more work should be done.

h. Oxygen uptake in the codling moth is increased 1 hr after exposure to UV-B. Oxygen uptake in young honey bee workers is not affected.

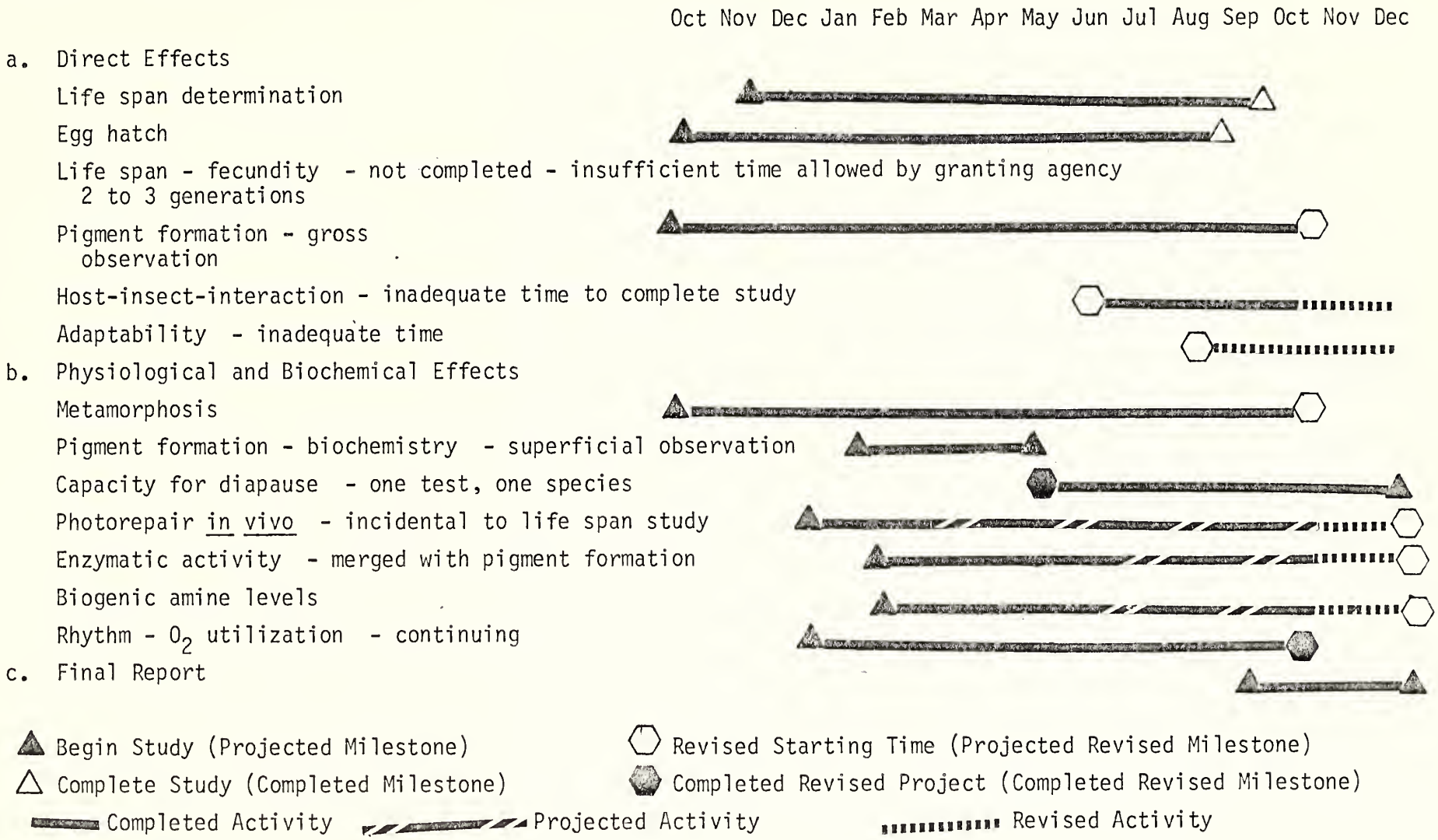
i. Infrared analysis of wash solutions from irradiated insects suggests that UV-B may solubilize some cuticular components.

RECOMMENDATIONS

If increased, UV-B will damage some insects at those stages of development during which these insects do not have adequate pigmentation to filter out harmful wavelengths. Honey bees and some other beneficial insects probably contain enough pigment to protect them. However, some eggs, larvae, pupae and adults of both beneficial and pest insects will be damaged if the cuticle contains little pigmented material or material which will absorb UV-B.

MILESTONES - EXTENT OF COMPLETION OF TASKS
REPORTING PERIOD JANUARY-DECEMBER 1977

Task VII. Influence of broad band UV-B on physiology and behavior of beneficial and harmful insects.



SECTION 3

EXPERIMENTAL PROCEDURES AND RESULTS

UV-B, EGG HATCHING AND LIFE SPAN IN THE PINK BOLLWORM

The effects of UV-B on the hatching of eggs of the pink bollworm supplied by the Animal and Plant Health Inspection Service Laboratory, USDA, Phoenix, Arizona, have been examined. Several different modes of exposure were employed. A preliminary test to determine effects of UV on hatchability indicates that FS-20 lamps filtered with either cellulose acetate or polystyrene inhibit egg hatching; 8 out of a minimum of 100 eggs hatched after exposure to UV-B between 9.349 mWm^{-2} BUV (3.1 suns; 0.97 Wm^{-2} Abs) - 13.34 mWm^{-2} BUV (4.4 suns; 1.38 Wm^{-2} Abs). Other early studies summarized in Tables 1 and 2 showed that high levels of UV-B reduced egg hatch. However, UV-B levels used were unrealistic in light of further work by IL and AEL, and the data shown in Table 3 indicate that continuous exposure to UV-B for 6 hr results in some inhibition of hatching at 9.349 mWm^{-2} BUV (3.1 suns; 0.97 Wm^{-2} Abs).

Apparently, insects exposed at "control" levels in the boxes used for UV exposure were in some instances (Boxes 1, 2, 4, and 5) exposed to enough UV-B so that hatching was inhibited.

Irradiation of pink bollworm eggs with UV-B did not affect incidence of diapause in larvae reared from these eggs. When larvae from one test were irradiated with 3.61 mWm^{-2} BUV (0.37 Wm^{-2} Abs) energy in the 280-330 nm region of the spectrum, adult survival in LD 16:8 as well as in two regimens in which the photophase was shifted, was affected. A mortality of 50% in the controls was observed 34 days after eggs were placed in the regimen LD 16:8 at 25°C. In irradiated insects 50% mortality was observed 21 days after insects were placed in LD 16:8 while in non-irradiated insects only 22% mortality was observed. In the irradiated insects rate of death decreased so that at slightly less than 50% mortality in control differences were not significant, while at slightly more than 50% mortality significant differences were observed. (See Table 4).

TABLE 1. EXPOSURE-PBW EGGS[a/] TO UV ON 12/6/76 (6 HR) and 12/7/76 + 12/8/76.

Treatment	Live larvae	Pupae	Adults	Empty vials	Dead larvae	Total empty vials + dead larvae	% survival	% early death	% late death	Total no.
Control[b/]	38	1	3	10	0	10	81	19		52
One 6 hr exposure - one day[c/]										
Box 1 - FS-20 bare lamp	4	1	1	2	0	2	75	25	0	8
Box 2 - FS-20 cellulose acetate filter	9	0	5	5	0	5	74	26	0	19
Box 3 - FS-20 plexiglass filter	14	1	1	5	0	5	76	24	0	21
Box 4 - FS-20 lamp	5	0	0	2	0	2	71	29	0	7
Two 6 hr exposures - one each day[c/]										
Box 1 - FS-20 bare lamp	0	0	0	3	0	3	0	100	0	3'
Box 2 - FS-20 cellulose acetate filter	19	0	6	15	0	15	63	37	0	40
Box 3 - FS-20 plexiglass filter	28	1	5	28	1	29	54	46	0	63
Box 4 - FS-20 lamp	1	0	0	1	0	1	50	50	0	2

Table 1 - Footnotes

a/ Filtered through polysterene.

b/ Control held at 24^{o} C LD 16:8. Control was not exposed to UV-B.

c/ Samples to be irradiated were placed 30 cm from the FS-20 lamps. Filters were used as indicated. Actual irradiance levels are not known.

TABLE 2. EXPOSURE PBW EGGS[a/] TO UV ON 12/9/76 - 6 HR EXPOSURE.

Treatment	Live larvae	Empty vials	Dead larvae	Total empty vials + dead larvae	% live	early death	late death	Total no.
Dark A	18	7	2	9	66.6	25.9	7.5	27
Dark C	15	6	1	7	68.2	27.2	4.5	22
Box 1 - FS-20 bare lamp	2	3	-	3	40.0	60.0	-	5
Box 2 - FS-20[b/] cellulose acetate filter	12	14	1	15	44.4	51.8	3.8	27
Box 3 - FS-20 plexiglass filter	53	24	6	30	64.1	28.9	7.0	83
Box 4 - FS-20 lamp	2	7	-	7	22.2	77.8	-	9

[a/] Eggs exposed 2 days after oviposition.

[b/] Energy level approximately 10.0 mWm^{-2} BUV (3.3 suns; 1.04 Wm^{-2} Abs) - 13.34 mWm^{-2} BUV (4.4 suns; 1.38 Wm^{-2} Abs) (see PSL report). Samples to be irradiated were placed 30 cm from the FS-20 lamps. Filters were used as indicated. Actual irradiance levels are not shown.

TABLE 3. HATCHING OF PINK BOLLWORM EGGS AFTER EXPOSURE TO VARIOUS LEVELS OF UV-B LIGHT

Box no.	Description	Irradiance mWm^{-2} BUV	SE[a/]	Wm^{-2} Abs	Set-up	No. hatched (3 hr)[b/]	No. hatched (6 hr)[b/]
1	FS-20 bare lamp	18.09	5.9	1.9	high	4	2
	(neutral density filter)	5.23	1.7	0.54	low	96	4
		-	-		control	40	10
2	FS-20 - polystyrene	10.59	3.5	1.1	high	72	4
		2.84	0.9	0.29	low	44	18
		-	-	-	control	52	12
3	G 15-T8 lamp	22.52	7.4	2.3	high	8	0
	254 line of UV spectrum	4.80	1.6	0.5	low	36	2
		-	-	-	control	60	30
4	FS-20 - cellulose acetate	10.38	3.4	1.1	high	27	12
	aged 6 hr	2.72	0.9	0.3	low	76	14
	5 mil	-	-	-	control	36	8
5	FS-20 - cellulose acetate	9.64	3.2	1.0	high	54	4
	aged 6 hr	2.17	0.7	0.2	low	44	8
	10 mil	-	-	-	control	28	4

[a/] Sun equivalents.

[b/] Numbers of eggs varied from 60-100. Pink bollworm females fix eggs to filter paper. We judged that it was deleterious to remove eggs from paper.

TABLE 4. MORTALITY OF PINK BOLLWORM AFTER IRRADIATION[a/] OF EGGS WITH UV-B LIGHT FOLLOWED BY DELAYS IN ONSET OF THE PHOTOPHASE[b/]

	Larvae found[b/]	Time from start to 50% mortality: Adults only days	Time from start to 50% mortality: Larvae, pupae, adults - days
CONTROL (LD 16:8)			
Not Irradiated	27	37	34
Irradiated	22	35	21[c/]
SHIFT-90° DELAY IN LD 16:8 EVERY			
3 DAYS	27	31	27
5	29	22[d/]	20[d/]
6	30	28[d/]	24[d/]
LL	28	34	33

[a/] 3.61 mWm^{-2} BUV (0.37 Wm^{-2} Abs) Energy - 280-320 nm; irradiated for 1 hr.

[b/] Temperature - 26°C; 40 vials per condition. Larvae found refers to larvae which developed at least to 3rd instar and were observed after infestation of medium.

[c/] After 50% mortality occurred, death rate decreased in irridiated insects so that when 48% mortality occurred in control, difference was not significant using chi-square but when 52% mortality was observed in controls, difference between control and irradiated was significant at <.025.

[d/] Significantly different from the control with P <.01 and .005 respectively using chi-square when mortality of 50% control mortality was determined.

EFFECTS OF UV-B ON MORTALITY OF THE HONEY BEE

Honey bee workers were exposed at the time in the life span during which they would normally first leave the hive.

The lamps were set up so that insects were irradiated at 7.32 mWm^{-2} BUV (0.76 Wm^{-2} Abs) with a 5 mil cellulose acetate filter and 2 FS-40 lamps 50 cm from the top of the carton containing the insects. When suitable holding conditions were obtained, in 3 tests, no differences in survival were observed between irradiated and test insects. No differences were detected when curves describing oxygen uptake as a function of time over a time span of 2 hr or less or 24 hr were inspected visually.

Lamp set up: 2 FS-40 lamps, insects 50 cm from lamp filter: Cellulose acetate, aged 6 hr 5 mil.

Expt 1 - single layer on lamps

Expt 2 - single layer on lamps

Expt 3 - single layer on lamps

Results are summarized in Tables 5, 6 and 7.

FIG. 1. CONTAINER FOR HONEY BEE TESTS

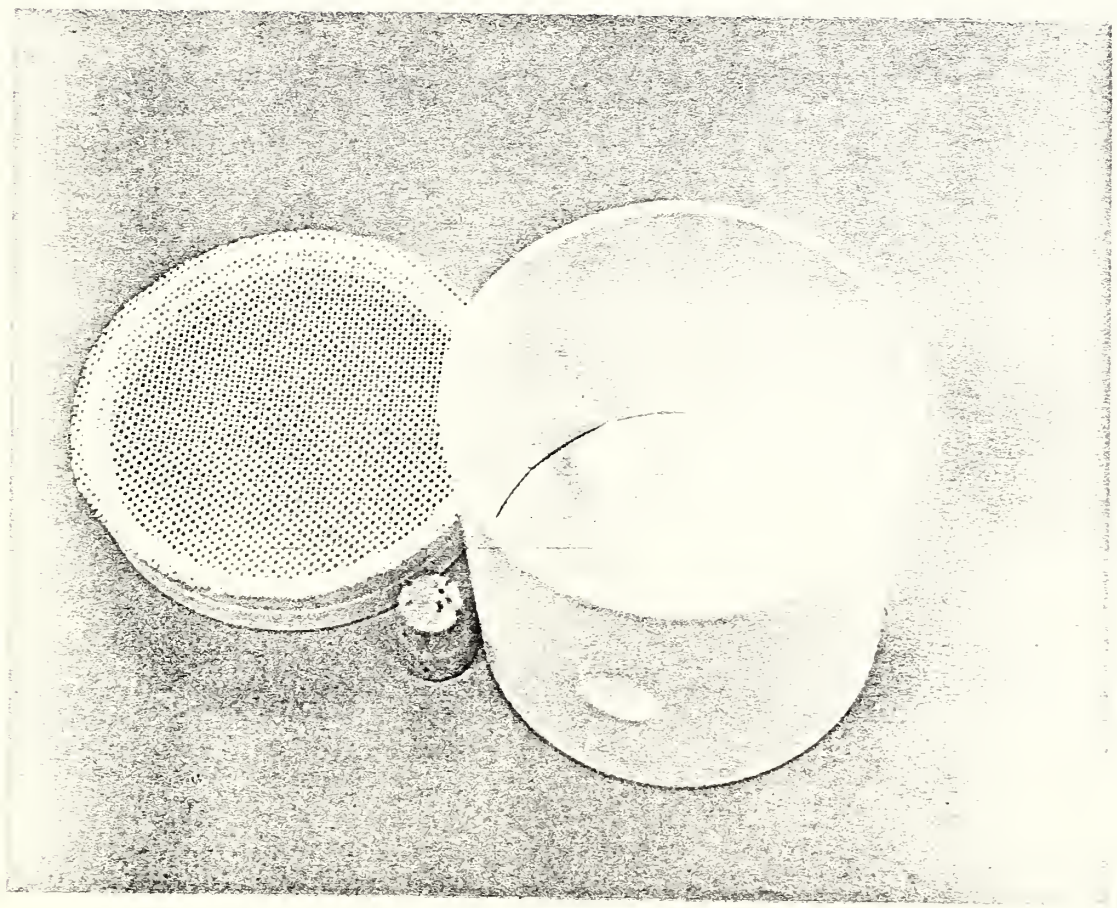


TABLE 5. UV-B AND HONEY BEE SURVIVAL

Experiment 1:

Honey bees (4-5 days) were received 6/27/77 and maintained with sugar cube and water in dish with styrofoam on top of water. 11 containers of bees each - 9 for UV and 2 for control in rear of room. (See Fig 1 for container)
Single layer 6 hr old CA filter (5 mil) on the lamps.
Irradiation - 6/28/77, 6 hr 11:00 - 5:00
Irradiation - 6/29/77 - 6 hr 11:00 - 5:00

Total Death Counts:

	Container No.	6/28	6/29	6/30	7/1	7/2	7/3	7/4	
Irradiated	1	0	1	1	1	3	5	8	
	2	0	1	1	1	6	8	10	
	3	0	0	0	0	5	9	14	
	4	0	0	0	4	4	5	6	
	5	0	0	1	3	4	5	5	
	6	0	0	0	2	2	3	4	
	7	0	1	1	4	6	9	10	
	8	0	0	0	0	1	2	3	
	9	0	0	0	1	1	2	2	
control	10	1	3	5[a/]	5	5	6	7	(mold in containers)
	11	0	0	0	0	4	6	9	

Conclusions: Initial death rate as high as first 4 experiments. Could be due to H_2O supply lasting longer. Note - even with lower mortality used only 1 layer CA/2 layers!

a/gummy with sugar.

TABLE 6. UV-B AND HONEY BEE SURVIVAL

Experiment 2:

Honey bees (4-5 days old) were received on 7/5/77 and maintained with sugar cube and water in dish - styrofoam on top of H_2O. 11 containers - 20 bees each, 9 for UV, 2 for control in rear of room.
Single layer of 6 hr old CA filter (5 mil) on lamp.
Irradiation - 7/6/77, 6 hr 10:00 - 4:00
Irradiation - 7/7/77, 6 hr 10:00 - 4:00

Total Death Counts:

	Container No.	7/6	7/7	7/8	7/9	7/10	7/11	7/12
Irradiated	1	0	0	0			0	0
	2	0	0	0	N	N	0	0
	3	0	0	0	0	0	0	0
	4	0	0	0	T	T	0	0
	5	0	0	0			1	4 (2 drowned)
	6	0	0	0	D	D	2	3
	7	0	0	1	0	0	2	2
	8	0	0	0	N	N	0	0
	9	0	0	0	E	E	0	0
control	10	0	0	0			0	0
	11	0	0	0			0	0

Conclusion: There was no initial death due to lamps.

TABLE 7. UV-B AND HONEY BEE SURVIVAL

Experiment 3:

Honey bees (4-5 days old) were received on 7/12/77 and maintained with sugar cube and water dish with styrofoam on top. 11 containers of bees with 20 bees each, 9 for UV-B and 2 for control in rear of room.
Single layer of 6 hr old CA (5 mil) on lamps
Irradiation - 7/12/77, 6 hr 11:00 - 5:00
Irradiation - 7/13/77, 6 hr 11:00 - 5:00

Total Death Counts:

	Control No.	7/12	7/13	7/14	7/15	7/18
Irradiated	1	0	0	1	1	1
	2	0	0	0	0	1
	3	0	0	0	2	2
	4	0	0	0	1	4
	5	0	0	0	3	5
	6	0	0	0	0	0
	7	0	0	0	0	3
	8	0	0	0	1	2
	9	1	1	1	1	1
control	10	0	0	0	1	3
	11	0	0	1	3	5

Conclusion: There was no initial mortality due to UV-B.

EFFECTS OF UV-B ON FACE FLY PUPAE

The effects of ultraviolet light on eclosion of face fly and house fly pupae 1-3 days after puparium formation were determined. Pupae were exposed to irradiances of approximately 6.68 mWm^{-2} BUV (2.25 suns; 0.69 Wm^{-2} Abs), 8.01 mWm^{-2} BUV (2.6 suns; 0.83 Wm^{-2} Abs), and 10.02 mWm^{-2} BUV (3.3 suns; 1.04 Wm^{-2} Abs). Light was supplied by 2 FS-40 tubes in one fixture fitted with a reflector prepared by AEL. Irradiances were determined by the IL with the Norris spectroradiometer; one large lot of polystyrene petri dishes was employed for both the irradiance measurements and the subsequent tests, since these dishes had been evaluated as filters during determinations of irradiance.

The UV-B light appeared to have no effect on eclosion of the house fly as shown in Table 8. In fact, exposure to UV-B may have enhanced emergence. Data such as those reported in Table 7 which indicate that UV-B had little effect on house fly eclosion, were obtained at least in 3 more experiments. However, the emergence of the face fly was affected by UV-B and perhaps the face fly could serve as biological dosimeter. When pupae of the face fly were exposed to approximately 8.01 mWm^{-2} BUV (2.25 suns; 0.69 Wm^{-2} Abs), (Table 9) eclosion was prevented in 30-50% of the insects. There was some variation from lot to lot, with some lots showing almost no emergence. Burned face fly pupae are illustrated in Figure 2. (Figure 2 also illustrated the pigmentation occurring when codling moth larvae are exposed to approximately 10.02 mWm^{-2} BUV (3.3 suns; 1.04 Wm^{-2} Abs to 13.36 mWm^{-2} BUV (4.4 suns; 1.38 Wm^{-2} Abs) with UV-B for a 6 hr span each day for 4 days.)

In further tests newly formed face fly pupae were irradiated by 5 intensities of UV-B to determine whether such exposures would increase pupal mortality or decrease adult fecundity, longevity, or egg viability. The tests were conducted by placing 100 newly-formed (<1 day old) pupae at each of 5 spots which were 32 cm beneath two 40-w BL fluorescent lamps which were covered by a sheet of 5 m thick cellulose acetate. The cellulose acetate was aged for 6 hr prior to any test of pupae and was replaced after every 18 hr of exposure to UV. Radiance was measured as fractions of a "standard sun" 3.06 mWm^{-2} BUV in weighed units. The pupae were held under 16 hr of cool white fluorescent lighting per day at $78^{o} \pm 2^{o}$ F and 40% ± 5% RH. Pupae were exposed each day for 3 days for periods of 0.25, 0.5, 1, 3, and 6 hr per day. (Table 10).

Exposure of pupae to 6.73 mWm^{-2} BUV (0.69 Wm^{-2} Abs) or more of UV-B radiation for periods of 1 hr per day for 3 days caused 77% pupal mortality and complete loss of adult fecundity (Tables 10 and 11, Fig. 3).

Since pupal exposure to UV-B over a 3-day period resulted in a high mortality, we exposed 4-day old pupae to UV-B for periods of 1, 2, 3, and 6 hr, during 1 day only, to see whether the developing gonads could be affected without killing the pupae. When the pupae were exposed for 3 hr or less, pupal mortality and adult fecundity were unaffected, but egg hatch was reduced by 25-41% (Table 12).

Future work will be directed towards using UV as an additional method of sterilizing male face flies.

TABLE 8. HOUSE FLY ECLOSION[a/] AFTER EXPOSURE TO UV-B[b/]

	No. eclosed	Avg.
Control	64	64
2.2 "suns"[c/]	98 78	88
2.6 "suns"[c/]	71 78	75
3.3 "suns"[c/]	85	85

a/ One hundred house fly pupae exposed in polystyrene petri dishes.

b/ January 10, 1977 run.

c/ Energy determined by AEL and IL.

TABLE 9. EMERGENCE OF ADULTS OF THE FACE FLY, MUSCA AUTUMNALIS, AFTER EXPOSURE OF PUPAE[a/] TO UV-B[b/]

Irradiance[c/]	% adult eclosion
Test 1 (1/14/77)	
Control	43 (33-53)
2.2 suns	67
2.6 suns	47
3.3 suns	14 (0-27)
Test 2 (2/1/77)	
Control	65
2.2 suns	64
2.6 suns	45.8
3.3 suns	24 (18-30)[d/]

a/ Five pupae/group, Test 1; 25 pupae/dish, Test 2. Single determinations run except where 2 values in parenthesis indicate duplication of test.

b/ UV-B supplied by 1 UV fixture equipped with a reflector and two FS-40 lamps. Cellulose acetate (5 mil).

c/ Irradiance in suns as described in text of report.

d/ Fifty insects in one dish.

TABLE 10. EFFECTS OF UV-B RADIATION (>265 nm) on face fly pupae - 1977

Duration of exposure	Pupal mortality (%) Standard suns[a/] 2.2	3.1	3.5	3.5	3.7	check	$\bar{x}$	Corrected mortality
6 hr for 3 days	98	90	95	95	93	28	94.2	92
3 hr " " "	98	98	100	99	100	22	99	98
1 hr " " "	83	81	84	77	87	22	82.4	77
1/2 hr " "	35	22	33	39	35	26	32.8	13
1/4 hr " "	12	14	26	19	13	19	16.8	0
$\bar{x}$	65.2	61.0	57.6	65.8	65.6	23.4		

[a/] 2.2 suns 6.73 mWm^{-2} BUV (0.69 Wm^{-2} Abs)

3.1 suns 9.35 mWm^{-2} BUV (0.97 Wm^{-2} Abs)

3.3 suns 10.02 mWm^{-2} BUV (1.04 Wm^{-2} Abs)

3.5 suns 10.69 mWm^{-2} BUV (1.10 Wm^{-2} Abs)

3.7 suns 11.35 mWm^{-2} BUV (1.17 Wm^{-2} Abs)

TABLE 11. EFFECTS OF UV-B RADIATION ON FACE FLY OVIPOSITION AND HATCH - 1977

	No. of eggs per female					% Hatch					
	Standard suns[a/]					Standard suns[a/]					
Duration of exposure	1.6	3.1	3.3	3.5	check	0.75	1.4	1.5	1.6	1.7	check
6 hr for each of 3 days	0	0	0	0	2						75
3 hr " " " " "	0	0	0	0	3						83
1 hr " " " " "	0	0	0	0	2.1						91
1/2 hr " " " " "	1.9	1.9	1.9	1.9	2	75	65	63	80	79	66
1/4 hr " " " " "	1.8	1.8	1.9	2.0	1.9	59	60	35	74	95	93

[a/] 1 sun = 3.06 mWm^{-2}

1.6 suns = 5.0 mWm^{-2} ABS (0.52 Wm^{-2} Abs)

3.1 suns = 9.35 mWm^{-2} BUV (0.97 Wm^{-2} Abs)

3.3 suns = 10.02 mWm^{-2} BUV (1.04 Wm^{02} Abs)

3.5 suns = 10.69 mWm^{-2} BUV (1.10 Wm^{-2} Abs)

TABLE 12. UV-B AS A STERILANT FOR FACE FLIES[a/]

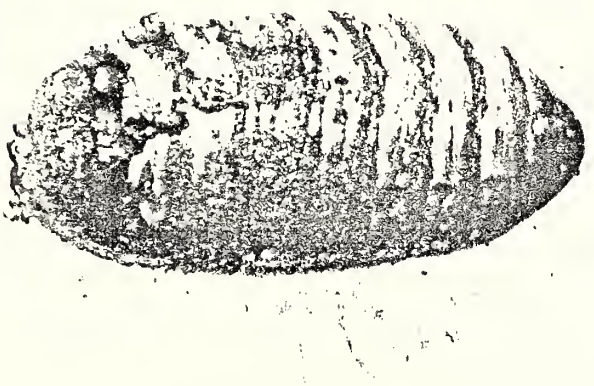
	No. of each stage after exposure to UV-B for:				
	1 hr	2 hr	3 hr	6 hr	0 hr
Pupae	100	100	100	100	100
Adults	97	100	94	95	100
Eggs	91	96	100	62	100
% Hatch	45	56	44	43	75
Corrected					
% mortality	40	25	41	43	

a/ Pupae were exposed to 11.3 mWm^{-2} BUV (3.7 suns; 1.17 Wm^{-2} Abs)

Fig. 2

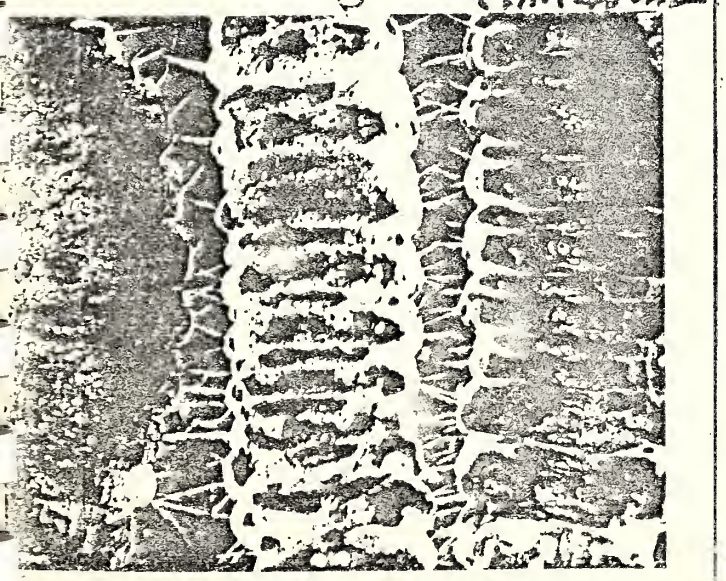
Slightly burned Face fly pupae

Normal tupiwred Face Fly pupae

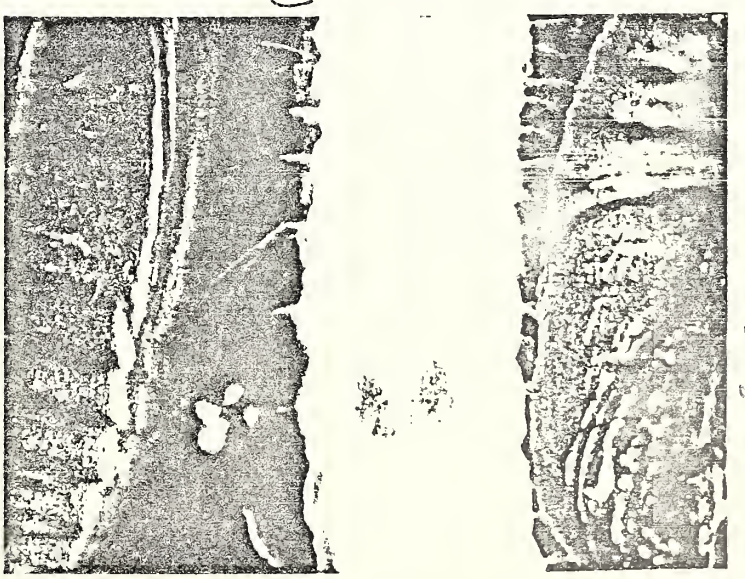


Badly burned Face fly pupae

Figure 1.

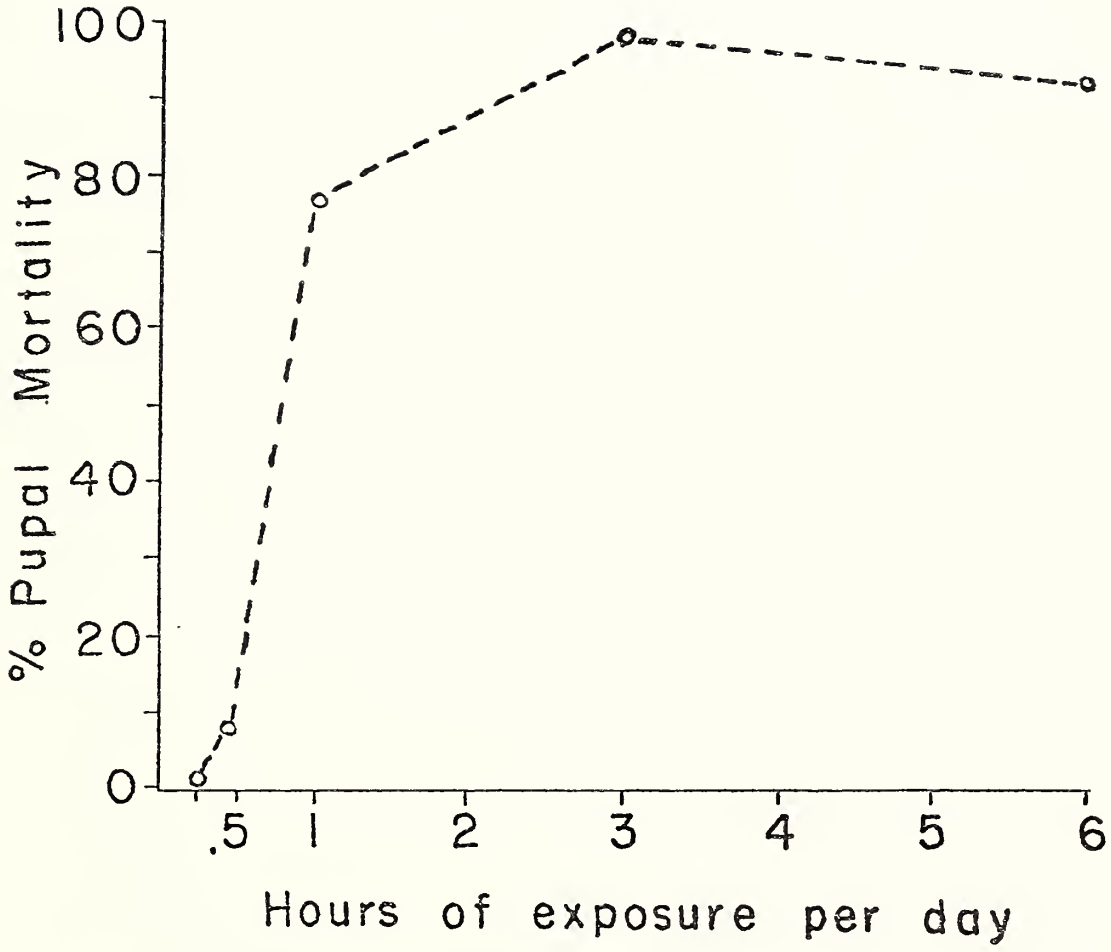


1 MIN.



1 Min C

Fig. 3



Effects of U.V. B on face fly pupae

PHOTOREPAIR IN VIVO

Possible occurrence of a repair enzyme was examined in one test, summarized in Table 13. In this test, larvae of the codling moth, the pink bollworm, and the European corn borer were divided into 3 groups, with one group serving as a control. A second group was exposed at a distance of 30 cm to two FS-40 lamps fitted with a mylar filter, and the third group to two FS-40 lamps fitted with a 5 mil cellulose acetate filter for 6 hr. After exposure, a portion of each group was held under room lights (about 4.5 uW/m^2), and a second portion was placed under two 15-watt daylight fluorescent lights at a distance of 6 cm.

TABLE 13. POSSIBLE REPAIR ENZYME ACTIVITY IN LARVAE OF TWO SPECIES OF LEPIDOPTERA

Insect	Control			Cellulose acetate		
	LD 16:8	Repair regimen	Darkness-no light	Control	Repair	Dark
Pink bollworm	1 pupa 1 larva 2 escaped	1 adult 1 pupa 3 larvae	1 adult 1 pupa 2 larvae 1 dead	1 larva 2 pupae 3 adults	1 adult 4 pupae	2 larvae 2 pupae 1 escaped
Codling moth	6 adults 1 deformed pupa 1 pupa 2 larvae egg masses present	5 adults 1 larva egg masses present	6 adults 3 deformed pupae 1 escaped egg masses present	5 adults 2 pupae 1 black larvae 2 dead larvae	1 deformed adult 4 adults 1 deformed pupa 3 pupae 1 dead larva	2 adults 1 egg mass 2 pupae 6 dead larvae

The European corn borer, Ostrinia nubilalis (HÜbner), larvae had crawled under dampened filter paper which had been provided as a moisture source and, thereby, escaped the UV radiation.

INSECT/PLANT INTERACTIONS

The major aspect of this phase of Task VII has been learning how to confine, irradiate and recover insects used in studies of insect-plant interaction. Trials with cages constructed of wire supports and nylon netting that completely surrounded infested bean plants in pots showed that this was not a satisfactory method of simultaneous exposure of plants and insects, since the insects escaped. At present the method of choice for small plants, such as soybeans and bush beans, is to place the pest insect, in this case the tobacco budworm, Heliothis virescens, on the plant in an aluminum and glass cage 26.5 x 26.5 x 26.5 mm with galvanized aluminum screen on top and sides.

Two FS-40 tubes in one fixture were placed 31 cm from the screen top of the cage. One thickness of 5 mil cellulose acetate was used as a filter. The screening, acting as a neutral density filter, absorbed about one third of the incident light. When 5 tobacco budworm larvae were placed on the one bean plant in the cage and the cage and its contents were exposed to UV-B during the last larval instar (about 4 days) at 25° C three survived and pupated and 2 died. Since the tobacco budworm is cannibalistic the 2 deaths may have resulted from injuries occurring during attacks of one insect on another. The 3 pupae were deformed and no adults emerged. In a control cage 2 larvae pupated and emerged as adults and 3 died. It is anticipated that some outdoor exposures can be conducted in these cages.

In further studies, when insects on plants were irradiated (5 plants) with 5 insects/plant) no differences were observed between control and test insect survival and qualitative observations suggested that irradiated insects were slightly more vigorous.

PIGMENT FORMATION AND ENZYMATIC ACTIVITY

Tests to determine effects of irradiation with UV-B of last instar codling moth larvae on subsequent *in vitro* activity of polyphenoloxidases were conducted by exposing test insects to UV-B at irradiance levels of approximately 9.349 mWm^{-2} BUV (3.1 suns; 0.97 Wm^{-2} Abs) or to 13.36 mWm^{-2} BUV (4.4 suns; 1.38 Wm^{-2} Abs) for 3, 2½, or 2 hr, homogenizing in (0.25M) sucrose in 0.1M phosphate buffer, pH 7.0, that was deoxygenated with UHP nitrogen for 5 min prior to placing insects in the solution.

Enzyme activity was measured by determining time until browning of a mixture of 0.1 ml of homogenate (0.3 g codling moth larva in 5 ml buffer) and 0.1 ml of 3, 4-dihydroxy-phenylalanine (3 mg/10 ml solution) as described by Hayes, Johnson, and Schechter (1975). Control homogenates were prepared from insects that were not irradiated.

Rates of browning differed on different days, but polyphenoloxidase activity was increased by exposure to UV-B. For example, a mixture prepared at 1552 hours from DOPA and homogenate from irradiated codling moths was observed to be blackened by 1645; the homogenate prepared from control insects required at least another hr to melanize.

When sensitivity to UV-B as a function of time of day was tested, we found that "browning" or melanization occurred more rapidly after 1 hr of irradiation (10.02 mWm^{-2} BUV (3.3 suns; 1.04 Wm^{-2} Abs)) at 1030 and 1130 than at 0830, 0930, 1230, and 1330 hr.

Analogous reactions may occur in tanning, or even sunburn in higher animals. It is likely that this kind of reaction in insects represents activation of polyphenoloxidase by injury. Such reactions occur when insects are cut or homogenized and in the studies reported here, rate of damage and possibly formation of autocatalytic products could occur in the intact, irradiated insect, as evidenced by Figure 1, in which 3 sunburned codling moth larvae which failed to pupate are illustrated. The companion figure shows portions of 2 pupae and 1 larva with the control larva appearing colorless and the pupae appearing normal in reproduction.

Attempts are being made to quantitate activity of this enzyme. Since oxygen is required for polyphenoloxidase activity, enhanced activity of this enzyme could be partially responsible for increased O_2 activity reported in some insects in another section of this report.

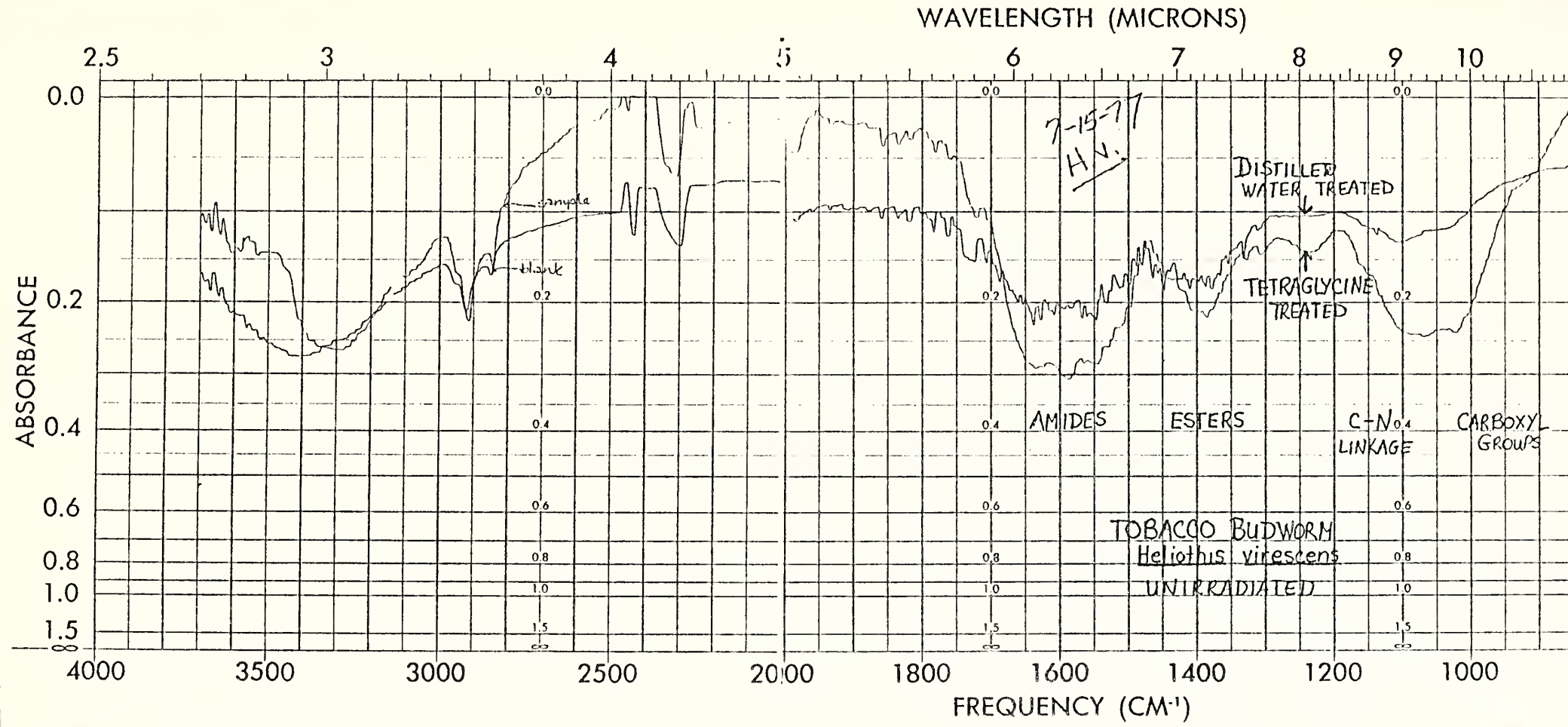
UV-B AND CHANGES IN THE INTEGUMENT OF LEPIDOPTERAN LARVAE

Pink bollworm larvae, codling moth larvae and European corn borer larvae were irradiated for 1 or 2 hr with UV-B at 7.32 mWm^{-2} BUV (2.4 suns; 0.76 Wm^{-2} Abs), using a 5 mil cellulose acetate filter and FS-40 lamps as already described.

Using a microliter syringe, 3 microliters of 0.01 M triglycine or tetraglycine was applied to the back of a larva to determine its effect and the effect of UV-B on the integrity of the integument. The droplet stayed on for five minutes and then was washed off with 0.1 ml of distilled water. Care was taken so that neither anterior nor posterior ends of the insect became wet. The insect was removed and .2 ml of acetone was added to stop the reaction. Distilled water served as a control.

This process was repeated three times using different larvae of the same age and species and the final solution (9 microliters triglycine, .3 ml distilled water, .6 ml acetone) was placed in a 10 ml centrifuge tube and then evaporated to dryness using a water bath (60 - 70° C) and a vacuum line. The crystals left behind after evaporation of the solvents were removed and made into a KBR disc for use in the Packard infrared spectrascope (IR).

The curves obtained by the controls showed that triglycine removed some substance (possibly fatty acid or wax) from the integument of each species tested. After UV-B treatment for one hour, the irradiated insects treated with distilled water showed a curve similar to that of a non-irradiated insect treated with triglycine and even more integumental material appeared in the wash solution. Typical IR curves are included. (Figs. 4a and 4b).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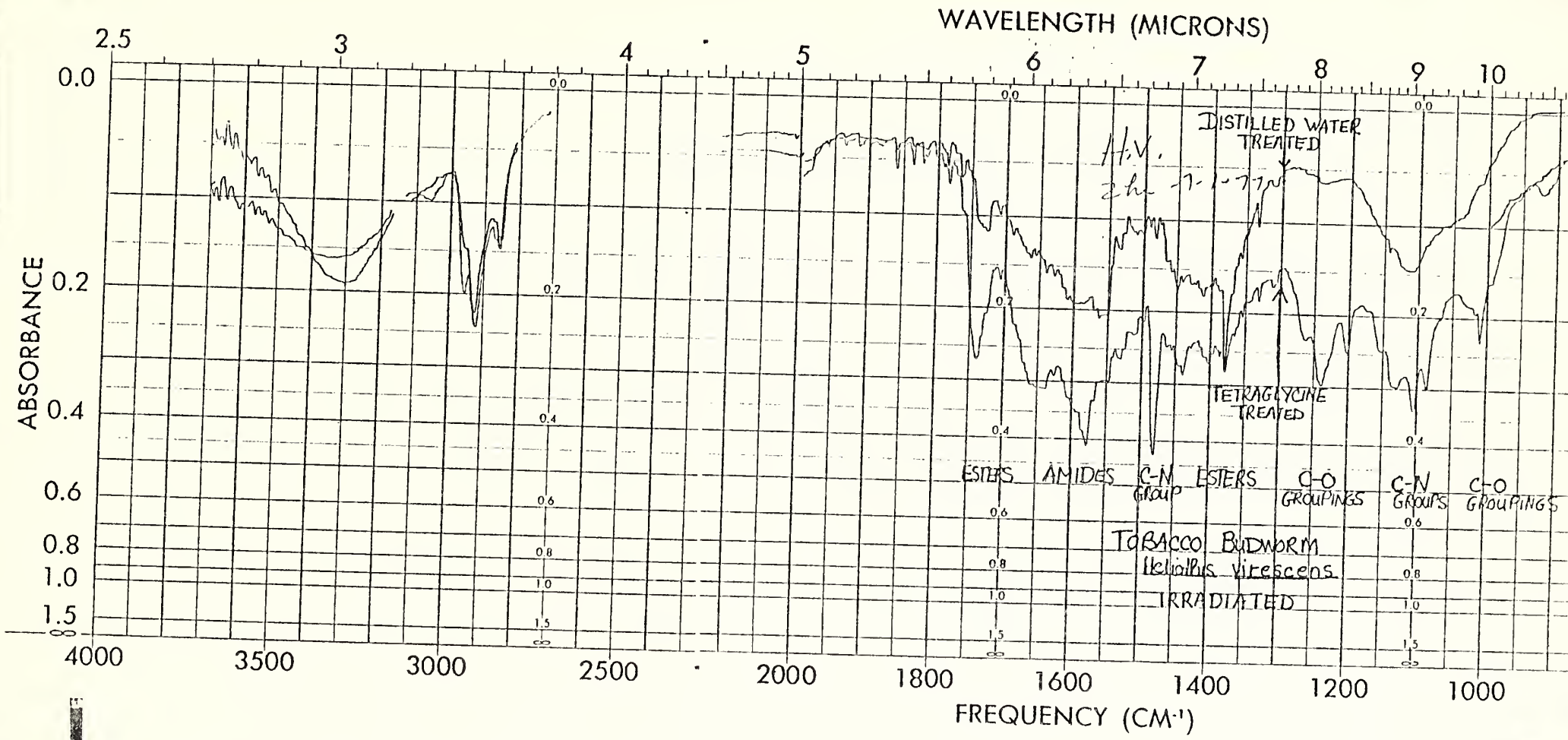
3
4
5
6
7
8
9
10
ABSORBANCE
0.0
0.2
0.4
0.6
0.8
1.0
1.5
∞
sample
blank
7-15-77
H.V.
DISTILLED WATER TREATED
TETRAGLYCINE TREATED
AMIDES
ESTERS
C-N LINKAGE
CARBOXYL GROUPS
TOBACCO BUDWORM
Heliothis virescens
UNIRRADIATED
4000
3500
3000
2500
2000
1800
1600
1400
1200
1000
FREQUENCY (CM-1)


WAVELENGTH (MICRONS)
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3
4
5
6
7
8
9
10
ABSORBANCE
0.0
0.2
0.4
0.6
0.8
1.0
1.5
∞
4000
3500
3000
2500
2000
1800
1600
1400
1200
1000
FREQUENCY (CM⁻¹)
DISTILLED WATER TREATED
H.V.
TETRAGLYCINE TREATED
ESTERS
AMIDES
C-N Group
ESTERS
C-O GROUPINGS
C-N GROUPS
C-O GROUPINGS
TOBACCO BUDWORM
Heliothis virescens
IRRADIATED

UV-B AND OXYGEN UPTAKE

In the determinations of the possible effects of UV-B on oxygen uptake, the insects used to date have been codling moth larvae, Laspeyresia pomonella (L.). Thinning apples were infested with eggs and shipped to Beltsville by the AR Laboratory, Yakima, Washington. When the insects arrived, they were maintained at 24^{0} C ± 1^{0} C under a lighting regimen of 16 hr light, 8 hr dark in a 24 hr day. The experiments were run with last instar larvae, 2 insects/run. The irradiated larvae were exposed for 2 hr periods to FS 40-40 watt ultraviolet lamps which had been aged 100 hr. Insects were held in polystyrene petri dishes which had been shown by the IL to transmit UV-B equivalent to 9.35 mWm^{-2} BUV (3.1 suns; 0.97 Wm^{-2} Abs) to 13.34 mWm^{-2} BUV (4.4 suns; 1.38 Wm^{-2} Abs) when suspended 15 cm above the insects. The control larvae were placed in the same room to receive the same background levels of exposure to white light (3 Wm^{-2}). After exposure, the larvae were placed in calibrated chambers and submerged in water at 24^{0} C. The experimental design is described by Hayes, Schechter, Mensins, and Horton (1968) and includes a polarographic electrode to measure the oxygen concentration. Minute by minute readings were collected from each larva and rate of oxygen uptake in µl O_2/mg/hr was calculated using an equation cited by Hayes. The Data System Application Division, AR, SEA, developed the program for determining O_2 uptake by insects and supplied the computer interface. The insects were allowed to equilibrate for the first hr; after that time, length of O_2 uptake determinations varied in time from 2 hr to 48 hr.

Figures 5a-d show that exposure for 1 hr to UV-B results in an initial increase of O_2 uptake and a subsequent damping of the short-term variations (ultradian rhythms) in oxygen utilization. This has been a characteristic pattern in the O_2 uptake of codling moth larvae exposed to UV-B. Such a pattern is probably also observed in other injured species. We can speculate that it represents, in part, the increased metabolic activity required to repair injuries.

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END: JULIAN DATE = 60 TIME = 910
INTERVAL: 5 MIN.
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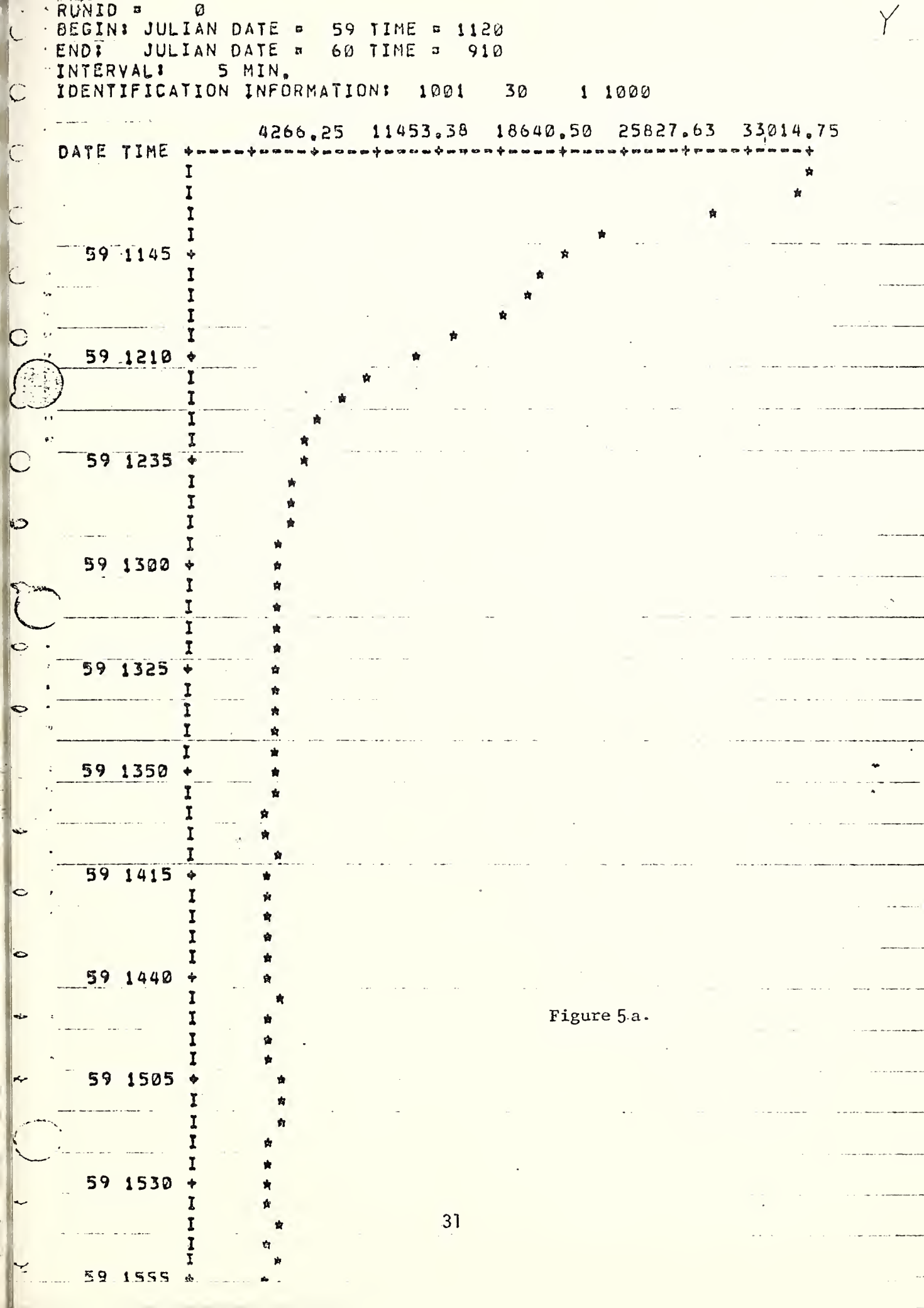

Figure 5.a.

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INTERVAL: 15 MIN.
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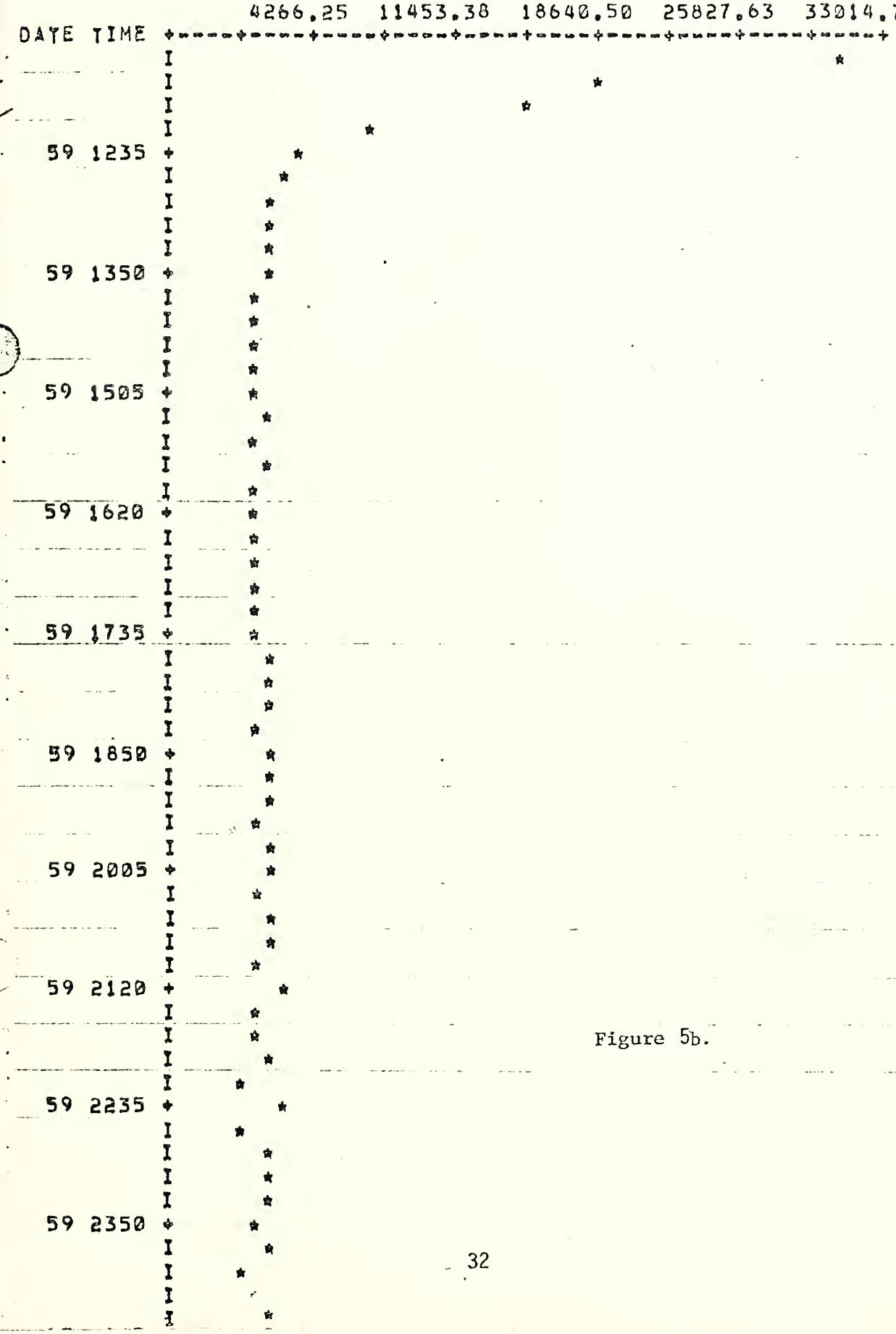

Figure 5b.

RUNID = 0
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END: JULIAN DATE = 60 TIME = 910
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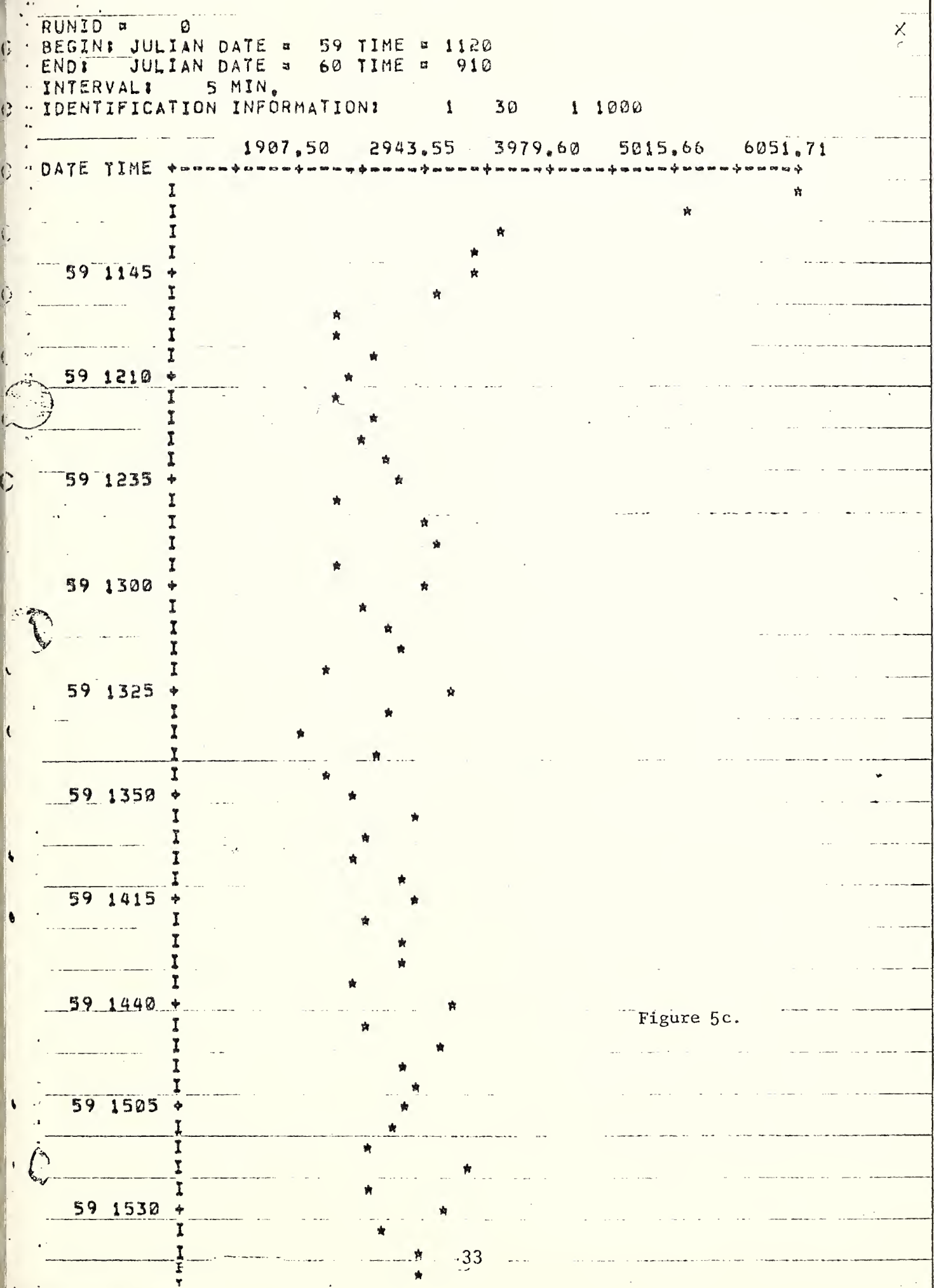
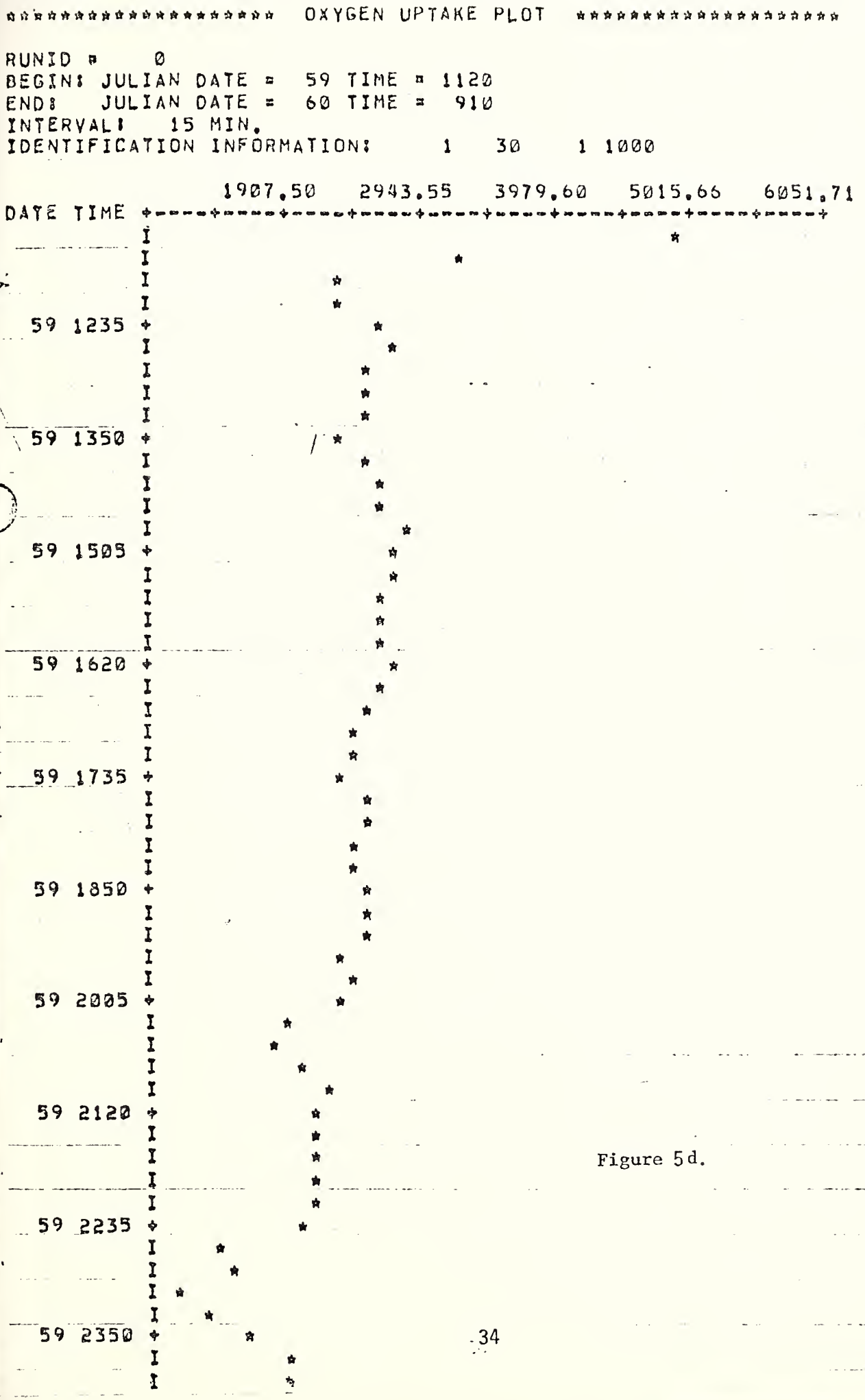


Figure 5c.


OXYGEN UPTAKE PLOT
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END: JULIAN DATE = 60 TIME = 910
INTERVAL: 15 MIN.
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DATE TIME
59 1235
59 1350
59 1505
59 1620
59 1735
59 1850
59 2005
59 2120
59 2235
59 2350

Figure 5d.

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